eye of *Limulus* the eccentric cell is joined to the individual retinular cells by electronic junctions; adjacent retinular cells are similarly joined (*2*, *4*). These findings indicate that all intraommatidial sensory cells are electrically coupled by relatively low-resistance electrotonic junctions. Thus, current injected into any one cell will flow into those cells to which it is coupled by such junctions (*2*). The rhabdomeric membranes of both retinular and eccentric cells have numerous cylindrical projections, the microvilli, which meet those of neighboring cells to form junctions, some or all of which may have low resistance (*5*). Consequently, currents transmitted from one cell to others presumably flow across a portion of the membrane system constituting the rhabdom. When a given retinular cell is hyperpolarized either directly or indirectly, the directions of current flow through the high-resistance portion of the retinular cell membrane to the extracellular space must be the same in both cases, since both procedures produce hyperpolarization; but the current flow through the relatively low-resistance electrotonic junctions must be of opposite directions in the two cases. Since hyperpolarizing currents injected directly into a retinular cell increase the latent period whereas hyperpolarizing currents injected into the eccentric cell or another retinular cell either shorten or do not change it, the direction of current flow through the electrotonic junctions in the rhabdom may be significant.

Modification of the latent period of the receptor potential of retinular cells by extrinsic currents is interesting because it demonstrates: (i) that the process which occurs during the latent period, a process which is initiated by light and determines when the receptor potential begins (*6*), can be influenced by extrinsic currents; (ii) that the direction of the current flow across some regions of the retinular cell membrane determines whether the latent period is increased; and (iii) that the rhabdomere of the retinular cell seems to be the site where the injected extrinsic current affects the latent period of the receptor potential.

Recently Lasansky and Fuortes (*7*) demonstrated that the microvillar membrane of the leech photoreceptor is the site of an inward, light-evoked current and concluded that the receptor potential originates in this structure. Our results also indicate that the rhabdomeric (microvillar) membrane generates the receptor potential. However, our data do not exclude the possibility that nonrhabdomeric membrane may also be actively involved (*2*, *8*, *9*).

The reduction in magnitude of the receptor potential produced by relatively strong hyperpolarizing currents may also depend on the direction of current flow through the rhabdomere. When hyperpolarizing currents are injected directly into a monitored retinular cell the magnitude of the receptor potential increases [(*2*, *8*) and Fig. 1]; if the currents are sufficiently strong, the magnitude decreases (*8*). Such reductions have been observed in seven experiments where it was expressly looked for; in one of these experiments, a hyperpolarizing current of 4.5 na increased the membrane potential by 64 mv and virtually suppressed the receptor potential. Yet, indirect hyperpolarization of retinular cells does not reduce the amplitude of the receptor potential (Fig. 2).

Our results support the conclusion that the receptor potential of the lateral eye of *Limulus* originates in retinular cells (*2*, *9*), that the rhabdomeric membrane system (or a part of this system) generates the receptor potential, and that the reaction or reactions occurring during the latent period may be influenced by intracellularly injected extrinsic currents.

V. J. WULFF, C. MENDEZ
Masonic Medical Research Laboratory, Utica, New York 13501

References and Notes

1. M. E. Behrens and V. J. Wulff, *J. Gen. Physiol.* **48**, 1081 (1965).
2. T. G. Smith and F. Baumann, *Progress in Brain Research* (Elsevier, Amsterdam, 1969), p. 313.
3. H. P. Reuben, R. Werman, H. Grundfest, *J. Gen. Physiol.* **45**, 243 (1961).
4. A. Borsellino, M. G. F. Fuortes, T. G. Smith, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 429 (1965); T. G. Smith, F. Baumann, M. G. F. Fuortes, *Science* **147**, 1446 (1965).
5. W. H. Miller, *Ann. N.Y. Acad. Sci.* **74**, 204 (1958); A. Lasansky, *J. Cell. Biol.* **33**, 365 (1967); W. H. Fahrenbach, *Z. Zellforsch.* **93**, 451 (1969); R. Whitehead and R. Purple, personal communication.
6. S. Hecht, *J. Gen. Physiol.* **1**, 657 (1919); V. J. Wulff, W. J. Fry, F. A. Linde, *J. Cell. Comp. Physiol.* **45**, 247 (1955).
7. A. Lasansky and M. G. F. Fuortes, *J. Cell. Biol.* **42**, 241 (1969).
8. R. Kikuchi and M. Tazawa, in *Electrical Activity of Single Cells* (Igakushoin, Tokyo, 1960), p. 25; R. Kikuchi, K. Naito, I. Tanaka, *J. Physiol.* **161**, 319 (1962).
9. T. H. Waterman and C. A. G. Wiersma, *J. Exp. Zool.* **126**, 59 (1954); T. Tomita, *Jap. J. Physiol.* **6**, 327 (1956).
10. Partly supported by NIH grant 5 R01 NB-03721-08. We thank W. J. Mueller, Director, Dept. of Bioelectronics and Computer Sciences, State University of New York, Upstate Medical Center, Syracuse, for his assistance, and Dr. R. Barlow, Sensory Communications Laboratory, Syracuse University, for his critical reading of this manuscript.

26 February 1970

Carbon-13 and Oxygen-18 in Dinosaur, Crocodile, and Bird Eggshells Indicate Environmental Conditions

Abstract. *We have gathered, from the nests of dinosaurs, and living and fossil birds, some evidence of the environment in which these creatures lived. However, our isotope determinations suggest it will be impossible to resolve the problem as to whether the dinosaurs were warm- or cold-blooded from the oxygen and carbon isotope content of their shells.*

In 1958 Russell speculated that dinosaurs might have been warm-blooded creatures unable, in their hairless state, to adapt to the changing environment of the late Cretaceous (*1*). Such speculation is recurrent, but difficult to test.

It occurred to us that a comparison of the oxygen and carbon isotopic composition of dinosaur eggshells from the Cretaceous beds at Shabarakh Usu, Mongolia, and near Aix in southern France, with those of calcareous eggshells of living reptiles (the crocodilians) and birds, living and extinct, might prove the dinosaurs to be more similar to one group than to the other.

Dinosaur eggshell fragments were collected (*2*) from Rousset near Aix, France, in 1960. We obtained shell fragments from the 1923 collections of the Central Asiatic Expedition near Shabarakh Usu in the Gobi desert, and a crocodile egg from the upper reaches of the Amazon basin in Peru (*3*). Thin-section and x-ray studies of the shell fragments suggested that the calcite comprising the dinosaur shells was not recrystallized nor contaminated by secondary carbonate and might well have retained its primary isotopic character. Various shell fragments, including those of the extinct giant bird of Madagascar, *Aepyornis* (*4*), chickens, ducks,

swallows, and shore birds were assembled for comparison.

The carbon and oxygen isotopes of the shell fragments were measured on a mass spectrometer. These measurements revealed excellent consistency of isotope data within shells taken from any one locality, but there appeared to be no isotopic relation in shell composition between the dinosaurs of southern France and those of the Gobi and no clear-cut isotopic grouping of birds, living cold-blooded reptiles, and dinosaurs. The similarity in the isotopic composition of eggs of quite different species from the same locality—for instance the large, plant-eating, dabbling ducks and the small caronymid-eating bank swallows of Lake Wabamun, Alberta, Canada—suggested the possibility that the isotopic composition of water ingested by the birds swamped subtler species-specific variations in the oxygen isotopic composition of the eggshells.

A controlled feeding experiment was set up with chickens fed a laying mash that was rich in carbohydrates and supplemented with limestone and drinking water of widely different isotopic compositions. The initial experiment established water as the critical variable, since variations in the isotopic composition of the carbonate supplement did not measurably affect the isotopic composition of the eggshells.

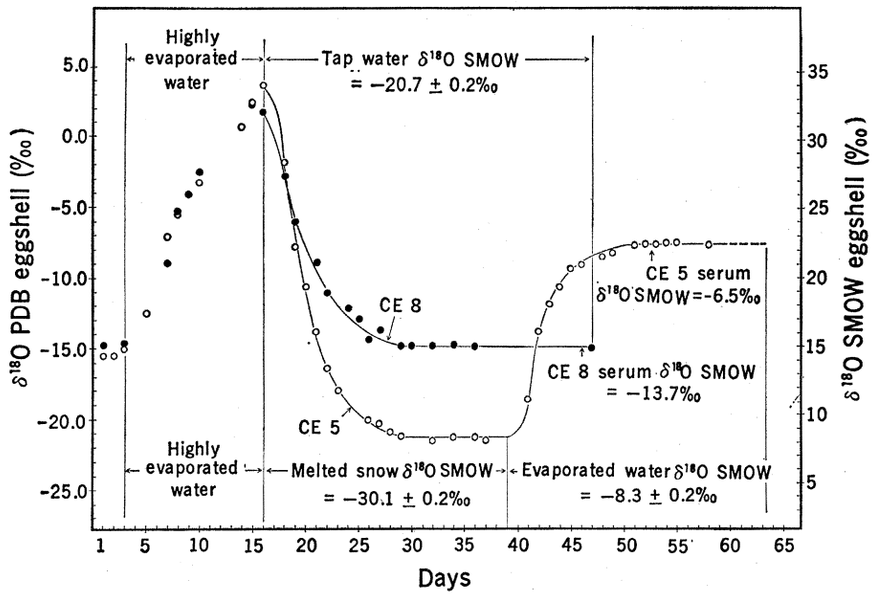

Fig. 1. Isotopic composition of chicken eggshells as influenced by diet with time.

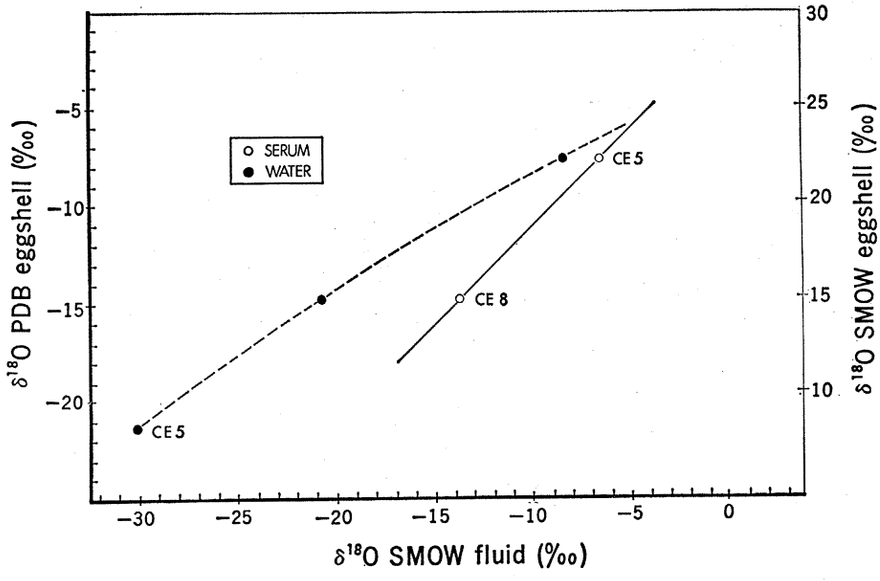

Fig. 2. Isotopic equilibrium in eggshells versus that in serum of chickens. Solid line represents isotopic equilibrium at 20.6°C or equilibrium at 107°F with biological fractionation factor.

Chickens that had been provided a diet of standard laying mash and tap water and that had been laying eggs with an isotopic composition of $\delta^{18}O \sim -15$ parts per thousand PDB were given access only to the same standard ration and highly evaporated water (Fig. 1). Over about 2 weeks this diet caused them to lay eggs progressively enriched in ^{18}O ($\delta^{18}O \sim +4$ parts per thousand PDB). On day 16, one chicken was placed on a diet of tap water and laying mash, and another was placed on a diet of melted snow (isotopically light) and laying mash. In about 2 weeks both were laying eggs with a constant isotopic composition that reflected their diets. The reaction was readily reversible.

The principal variable in producing different oxygen isotopic composition in eggshells is the water available to the bird. Only a limited amount of data on blood serum was collected during the experiments (since collection interrupted the laying sequence), and no determinations of the isotopic composition of the laying mash (mostly cereal grains produced on parkland) were made. However, limited data show a general increase in content of ^{18}O in the blood serum reflected in an increase in content of ^{18}O with the eggshell (Fig. 2). Apparently, some of the oxygen in the eggshell is contributed by the atmospheric oxygen of respiration and the carbohydrate oxygen of the laying mash (Fig. 2) of about $\delta^{18}O$ −5 parts per thousand SMOW.

There is an approximately linear relation of oxygen in the carbonate of the eggshell to the ^{18}O content of the water ingested (Fig. 3). We have no direct information on the nature of the water available to the dinosaur *Hypselosaurus* from southern France nor to *Protoceratops* from the Gobi. Paleogeographic maps suggest that *Hypselosaurus* lived on the latest Cretaceous Durancian isthmus (*5*). Paleolatitude was probably about 25°N, about the same as the latitude in which *Aepyornis* of Madagascar lived (25°S), and the paleoecologic setting was probably remarkably similar to that of present-day Madagascar (*4*). In this coastal setting of strong evaporation and seasonal rainfall most of the water available was

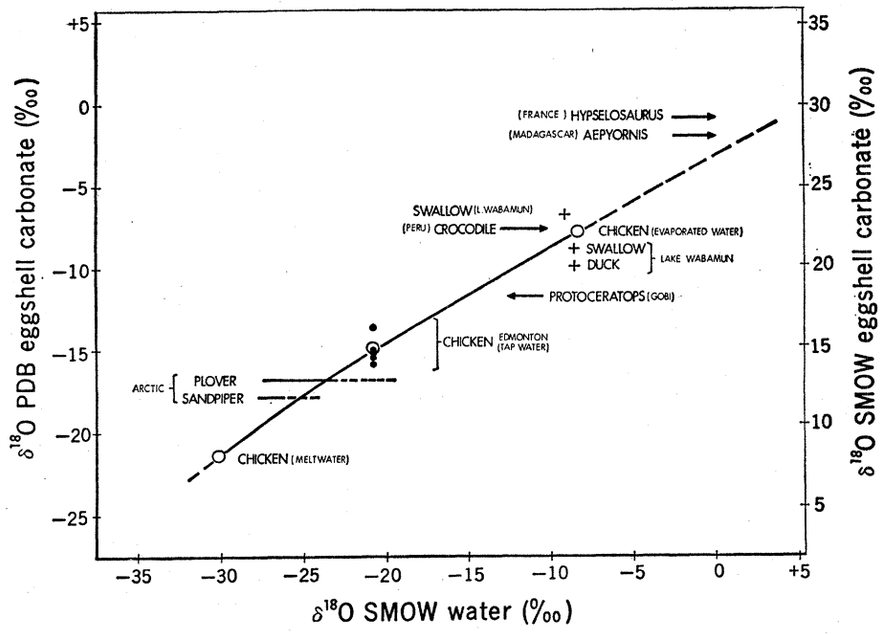

Fig. 3. Oxygen isotopic composition of eggshell carbonate in reptiles and birds as a function of that in drinking water.

probably the relatively isotopically heavy first precipitation from evaporated seawater [at Tananarive, Madagascar, −7 parts per thousand SMOW (*6*)]. One might expect to find *Hypselosaurus* and *Aepyornis* eggshells comparatively enriched in ^{18}O, and such is the case.

On the other hand, *Protoceratops* of the early Upper Cretaceous of Mongolia lived in an association with crocodiles and tortoises on the continental delta of an enormous ancient river fed by continental water coming off the rising highlands (*7*). These continental waters from the cool highlands would have been much lighter (isotopically) than those of Madagascar or the Cretaceous of the low Durancian isthmus and might be expected to have been comparable with those available to the crocodile of Peru, in the upper reaches of the Amazon, a river fed by isotopically light waters off the eastern slope of the Andes. The eggs indicate that such is the case.

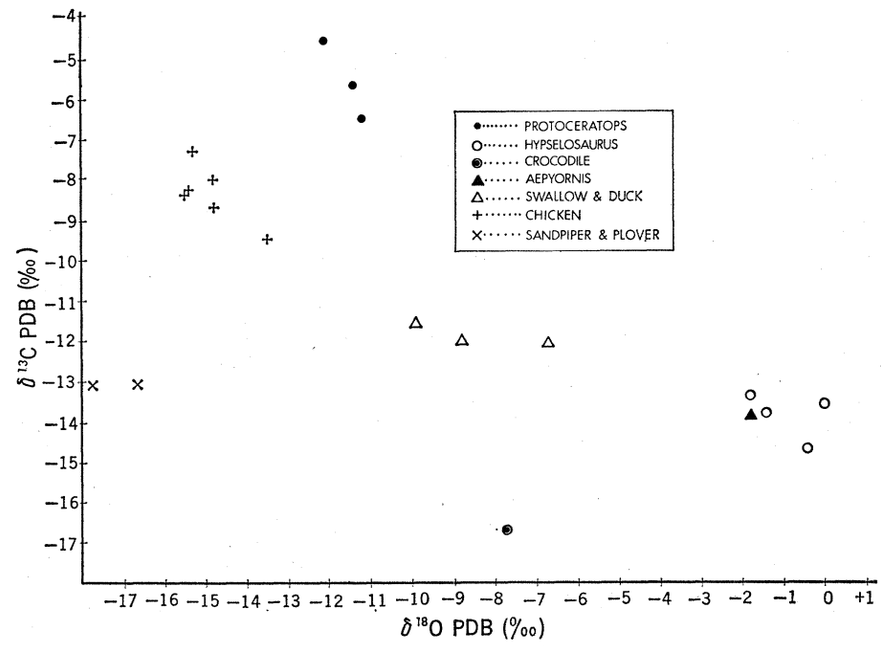

Fig. 4. Carbon isotopic composition versus oxygen isotopic composition in eggshells of birds and reptiles.

The eggs of *Protoceratops* and the Peruvian crocodile are not dissimilar in ^{18}O content to those of bank swallows and dabbling ducks, living on the shores of Lake Wabamun, a large lake west of Edmonton, Alberta (where precipitation ranges from $\delta^{18}O$ of −11 to −26 parts per thousand SMOW). Lake Wabamun, in a continental environment, has a strong evaporitic regime with no active surface outlet and somewhat isotopically heavier water than the local precipitation ($\delta^{18}O \sim -9$ parts per thousand SMOW).

In high latitude environments of the Canadian Arctic where the lower temperature results in lighter $\delta^{18}O$ precipitation, shore birds living near meltwater ponds lay eggs depleted in ^{18}O, which are comparable to those of the chicken fed on melted snow.

Carbon isotope studies of all the shells (Fig. 4) may provide a little paleoenvironmental information. Carbon isotopes fractionate during photosynthetic formation of amino acids (*8*). Present-day land plants have less ^{13}C than marine plants (*9*). *Protoceratops* and *Hypselosaurus* are quite different, *Protoceratops* has the most heavy carbon. Carbon isotopes from *Hypselosaurus* and *Aepyornis* eggshells are isotopically much lighter and similar (confirming the similarity in paleoecologic environment), and the carbon from the Peruvian crocodile egg is the lightest. Perhaps the tropical rain forest favors light carbonate, as do oceanic environments, arctic to tropical.

Dryer continental environments seem to favor heavier carbonate, chickens fed with cereal grains growing on parkland produce eggs with more ^{13}C than do birds from the same place consuming foods grown in water (swallow, duck). The food available to *Protoceratops* (usually depicted as existing in a semi-arid environment) produced the heaviest carbon. It is unfortunate that dinosaur egg fragments are not preserved in the lime-poor continental Cretaceous beds of western North America, where from studies of the oxygen isotope content of nearby marine fossils more can be inferred about paleoenvironment (*10*).

R. E. Folinsbee, P. Fritz
H. R. Krouse, A. R. Robblee
Departments of Geology, Physics, and Animal Science, University of Alberta, Edmonton 7, Alberta, Canada

References and Notes

1. L. Russell (Royal Ontario Museum) speaking at a lecture to celebrate opening the biological science wing at the University of Alberta.
2. A. F. de Lapparent, *C. R. Acad. Sci. Paris* **245**, 546 (1967).
3. We thank E. H. Colbert (American Museum of Natural History) for the specimens.
4. M. L. Marsden, *Nat. Geogr. Mag.* **132**, 443 (1967); A. Wetmore, *ibid.*, p. 448.
5. M. Gignoux, *Stratigraphic Geology* (Freeman, San Francisco, 1955).
6. W. Dansgaard, *Tellus* **16**, 436 (1964).
7. E. H. Colbert, *Men and Dinosaurs* (Dutton, New York, 1968); R. Brinkmann, *Abriss der Geologie* (Enke, Stuttgart, 1959).
8. P. H. Abelson and T. C. Hoering, *Proc. Nat. Acad. Sci. U.S.* **47**, 623 (1961).
9. S. R. Silverman and S. Epstein, *Bull. Amer. Ass. Petrol. Geol.* **42**, 998 (1958).
10. H. A. Tourtelot and R. O. Rye, *Bull. Geol. Soc. Amer.* **80**, 1903 (1969).

11. Supported by National Research Council of Canada and the University of Alberta research fund. We thank R. Fox (University of Alberta) for reading the manuscript, A. F. de Lapparent for advice, and C. R. Stelck for criticism and for the fragments of *Aepyornis* eggshells from the Department of Geology Museum, University of Alberta, Edmonton, Alta., Canada.

19 February 1970

Chromosomal Localization of Mouse Satellite DNA

Abstract. *Hybridization of radioactive nucleic acids with the DNA of cytological preparations shows that the sequences of mouse satellite DNA are located in the centromeric heterochromatin of the mouse chromosomes. Other types of heterochromatin in the cytological preparations do not contain satellite DNA.*

One characteristic which distinguishes the DNA of higher organisms from that of bacteria is the presence of families of repeated nucleotide sequences. These repeated sequences are found in multiplicities ranging from 10^2 to 10^6 per genome (*1*), but very little is known about their function or their organization within the chromosomal complement. Recently a technique which makes possible the cytological localization of specific nucleotide sequences has been developed (*2*). This localization is accomplished by hybridizing the DNA of cytological preparations with radioactive nucleic acid. The regions in the preparation to which the radioactive nucleic acid has bound are then detected by autoradiography. Such a technique permits a direct investigation of the distribution of families of repeated sequences within the genome.

Perhaps the most thoroughly studied fraction of repetitive DNA, with the exception of the sequences coding for ribosomal RNA, is the mouse satellite DNA. Therefore we chose mouse satellite DNA for our first investigations of the cytological localization of multiply repeated DNA sequences. We show that this fraction is located in the centromeric heterochromatin of the mouse chromosomes, a fact which we have briefly reported (*3*).

Mouse satellite DNA forms a band slightly separated from the main peak when mouse DNA is spun to equilibrium in a CsCl density gradient (*4*). It makes up about 10 percent of the total DNA, regardless of the tissue from which the DNA has been prepared, and is found in about the same proportion in tissue culture lines (*5*). From renaturation kinetics it has been estimated that mouse satellite DNA consists of approximately 10^6 copies per genome of a sequence some 400 nucleotide pairs in length (*6*). It is possible that the copies are not all identical. However, the rapid reassociation seen after denaturation indicates a high degree of homogeneity (*6*). Although the sequences of the mouse satellite make up 10 percent of the mouse DNA they do not seem to code for a corresponding fraction of the RNA in the tissues which have been studied. Flamm, Walker, and McCallum (*7*) were unable to detect any hybridization of satellite DNA to RNA from mouse liver, spleen, or kidney. Recently Harel *et al.* (*8*) have reported that rapidly labeled RNA from some of the same tissues did bind to satellite DNA. The coding properties of this fraction require further study.

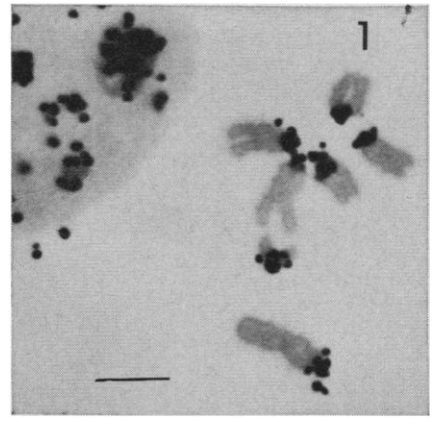

Fig. 1. Autoradiograph of a mouse tissue culture preparation after cytological hybridization with radioactive RNA copied in vitro from mouse satellite DNA. The RNA has bound to the centromeric heterochromatin of the chromosomes and to the chromocenters of the interphase nucleus on the left. The DNA of this preparation was denatured in situ by treatment with 0.07*N* NaOH. The slide was then incubated with radioactive RNA for 10 hours at 66°C. The preparation was treated with ribonuclease to remove RNA that was not specifically bound and then coated with autoradiographic emulsion. The RNA had a calculated specific activity of 7×10^7 disintegrations min^{-1} μg^{-1}. Slide stained with Giemsa. Exposure, 5 days; × 2000; scale length, 5 μm.

Our hybridization experiments on the localization of mouse satellite DNA have been done in two ways. First, we have applied fractions of radioactive mouse DNA to cytological preparations of mouse tissue culture cells (*9*). The radioactive DNA was extracted from tissue cultures of the mouse A9 line grown in medium containing [^{3}H]thymidine (*3*). Satellite DNA was separated from the rest of the mouse DNA by silver-ion–cesium sulfate (*10*) density gradient centrifugation. The DNA was denatured with heat before use in the hybridization reaction. This DNA had a specific activity of 200,000 cpm μg^{-1} as determined by spotting known amounts of the DNA on a nitrocellulose filter and counting the filter in toluene fluor in a scintillation counter. In the second type of experiment we applied radioactive RNA, transcribed in vitro from mouse DNA, to cytological preparations of both mouse testis and mouse tissue culture (*2*). Mouse liver DNA was fractionated by silver-ion–cesium sulfate centrifugation. The satellite DNA and the main peak DNA were transcribed separately with *Escherichia coli* RNA polymerase (*11*) and tritiated ribonucleotide triphosphates. This complementary RNA had a calculated specific activity of 7×10^7 disintegration $min^{-1} \mu g^{-1}$. In all experiments the DNA of the cytological preparations was denatured by treatment with NaOH before hybridization. Autoradiographs were exposed for several days when hybridization was done with complementary RNA and for several months when hybridization was done with radioactive DNA.

The normal mouse chromosomal complement consists of 20 pairs of telocentric or acrocentric chromosomes (*12*). Each chromosome has a region next to the centromere which can be identified as heterochromatin by its staining properties. In some mouse tissue culture lines a few of the chromosomes are metacentric and have presumably arisen by fusion of two of the chromosomes of the normal com-